PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C07D 498/18, A61K 31/445 // (C07D 498/18, 311:00, 273:00 C07D 221:00)

A1

(11) International Publication Number: WO 93/18043

(43) International Publication Date: 16 September 1993 (16.09.93)

(21) International Application Number: PCT/US93/01863

(22) International Filing Date: 3 March 1993 (03.03.93)

(30) Priority data:
07/846,637 5 March 1992 (05.03.92) US
9223760.1 12 November 1992 (12.11.92) GB

(71) Applicant: AMERICAN HOME PRODUCTS CORPORATION [US/US]; 685 Third Avenue, New York, NY 10017 (US).

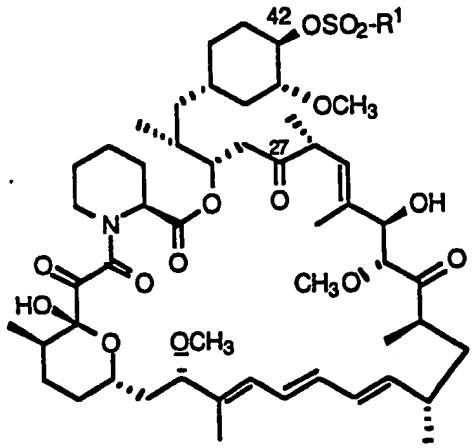
(72) Inventors: FAILLI, Amedeo, Arturo ; 14 Landing Lane, Princeton Junction, NJ (US). KAO, Wenling ; 1742 Jennings Way, Paoli, PA (US). STEFFAN, Robert, John ; 263 Wheatsheaf Lane, Langhorne, PA (US). VOGEL, Robert, Lewis ; 20 Sleepy Hollow Road, Stratford, NJ (US).

(74) Agents: ALICE, Ronald, W.; American Home Products Corporation, 685 Third Avenue, New York, NY 10017 (US) et al.

(81) Designated States: AU, BB, BG, BR, CA, CZ, FI, HU, JP, KP, KR, KZ, LK, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, UA, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG).

Published
With international search report.

(54) Title: NOVEL RAPAMYCIN 42-SULFONATES AND 42-(N-CARBOALKOXY)SULFAMATES USEFUL AS IMMUNOSUPPRESSIVE AGENTS



(I)

(57) Abstract

The invention concerns compounds of formula (I) where R^1 is alkyl, alkenyl, or alkynyl containing 1 to 6 carbon atoms; or an aromatic moiety selected from phenyl, naphthyl and 4-(phenylaza) phenyl wherein said aromatic group is optionally substituted by one or more substituents selected from C_1-C_6 alkyl, C_1-C_6 alkoxy, hydroxy, amino, mono- or di-(C_1-C_6)alkylamino, carboxy, (C_1-C_6 alkoxy)carbonyl, C_2-C_7 alkanoyl, (C_1-C_6) thioalkyl, halo, cyano, nitro, trifluoromethyl, trifluoromethoxy or R^1 is a heteroaromatic group of 5 to 10 ring atoms containing oxygen, nitrogen and/or sulfur as heteroatom(s) wherein said heteroaromatic group is optionally substituted by one or more substituents selected from C_1-C_6 alkyl, C_1-C_6 alkoxy, hydroxy, amino, mono- or di-(C_1-C_6)alkylamino, carboxy, (C_1-C_6 alkoxy)carbonyl, C_2-C_7 alkanoyl, (C_1-C_6) thioalkyl, halo, cyano, nitro, trifluoromethyl, trifluoromethoxy or R^1 is $NHCO_2R^2$ wherein R^2 is lower alkyl containing 1 to 6 carbon atoms; or a pharmaceutically acceptable salt thereof, which compounds are useful in the treatment of transplantation rejection, host versus graft disease, autoimmune diseases and diseases of inflammation.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NL	Netherlands
BE	Belgium	GN	Guinea	NO	Norway
BF	Burkina Faso	GR	Greece	NZ	New Zealand
BG	Bulgaria	HU	Hungary	PL	Poland
BJ	Benin	IE	Ireland	PT	Portugal
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SK	Slovak Republic
CI	Côte d'Ivoire	KZ	Kazakhstan	SN	Senegal
CM	Cameroon	LI	Liechtenstein	SU	Soviet Union
CS	Czechoslovakia	LK	Sri Lanka	TD	Chad
CZ	Czech Republic	LU	Luxembourg	TG	Togo
DE	Germany	MC	Monaco	UA	Ukraine
DK	Denmark	MG	Madagascar	US	United States of America
ES	Spain	ML	Mali	VN	Viet Nam
FI	Finland	MN	Mongolia		

NOVEL RAPAMYCIN 42-SULFONATES AND 42-(N-CARBOALKOXY)SULFAMATES USEFUL AS IMMUNOSUPPRESSIVE AGENTS

BACKGROUND OF THE INVENTION

This invention relates rapamycin 42-sulfonates and a method for using them in the treatment of transplantation rejection, host versus graft disease, autoimmune diseases, diseases of inflammation, and fungal infections.

Rapamycin is a macrocyclic triene antibiotic produced by Streptomyces hygroscopicus, which was found to have antifungal activity, particularly against Candida albicans, both in vitro and in vivo [C. Vezina et al., J. Antibiot. 28, 721 (1975); S. N. Sehgal et al., J. Antibiot. 28, 727 (1975); H. A. Baker et al., J. Antibiot. 31, 539 (1978); U.S. Patent 3,929,992; and U.S. Patent 3,993,749].

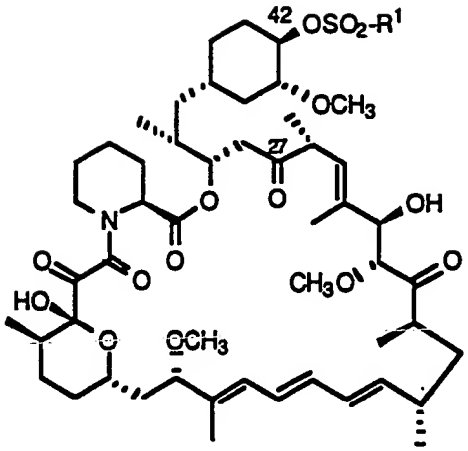
Rapamycin alone (U.S. Patent 4,885,171) or in combination with picibanil (U.S. Patent 4,401,653) has been shown to have antitumor activity. R. Martel et al. [Can. J. Physiol. Pharmacol. 55, 48 (1976)] disclosed that rapamycin is effective in the experimental allergic encephalomyelitis model, a model for multiple sclerosis; in the adjuvant arthritis model, a model for rheumatoid arthritis; and effectively inhibited the formation of IgE-like antibodies.

The immunosuppressive effects of rapamycin have been disclosed in FASEB 3, 3411 (1989). Rapamycin has been shown to be effective in inhibiting transplant rejection (U.S. Patent Application Ser. No. 362,544 filed June 6, 1989). Cyclosporin A and FK-506, other macrocyclic molecules, also have been shown to be effective as immunosuppressive agents, and therefore also useful in preventing transplant rejection [FASEB 3, 3411 (1989); FASEB 3, 5256 (1989); and R. Y. Calne et al., Lancet 1183 (1978)].

Mono- and diacylated derivatives of rapamycin (esterified at the 28 and 43 positions) have been shown to be useful as antifungal agents (U.S. Patent 4,316,885) and used to make water soluble prodrugs of rapamycin (U.S. Patent 4,650,803). Recently, the numbering convention for rapamycin has been changed; therefore according to Chemical Abstracts nomenclature, the esters described above would be at the 31- and 42-positions.

DESCRIPTION OF THE INVENTION

This invention relates to rapamycin 42-sulfonates and 42-(N-carboalkoxy)sulfamates of general formula (I)



(I)

where R^1 is C_1-C_6 alkyl, C_1-C_6 haloalkyl, C_2-C_6 alkenyl, or C_2-C_6 alkynyl or an aromatic moiety selected from phenyl, naphthyl and 4-(phenylaza)phenyl or a heteroaromatic group of 5 to 10 ring atoms containing oxygen, nitrogen and/or sulfur as heteroatoms(s) wherein said aromatic or heteroaromatic group is optionally substituted by one or more substituents selected from C_1-C_6 alkyl, C_1-C_6 alkoxy, hydroxy, amino, mono- or di(C_1-C_6) alkylamino, carboxy, (C_1-C_6 alkoxy)carbonyl, C_2-C_7 alkanoyl, (C_1-C_6) thioalkyl, halo, cyano, nitro, trifluoromethyl, trifluoromethoxy; or R^1 is $NHCO_2R^2$ wherein R^2 is lower alkyl containing 1 to 6 carbon atoms; or a pharmaceutically acceptable salt thereof.

Examples of alkyl for R^1 are straight or branched chain groups preferably of 1 to 4 carbon atoms such as methyl, ethyl, propyl and butyl.

Examples of haloalkyl are trifluoromethyl and 2,2,2-trifluoroethyl.

Examples of alkenyl are vinyl, alkyl, prop-1-enyl and but-2-enyl.

Examples of alkynyl are ethynyl and prop-2-ynyl.

Examples of aromatic and heteroaromatic groups for R^1 are phenyl and naphth-1-yl, thienyl (e.g. thien-2-yl); furyl (e.g. furan-2-yl), pyridyl (e.g. pyrid-4-yl),

quinolyl (e.g. quinol-8-yl) and isoquinolyl (e.g. isoquinol-4-yl). Examples of substituents for aromatic or heteroaromatic R^1 groups are: straight or branched chain alkyl preferably of 1 to 4 carbon atoms such as methyl, ethyl, propyl or butyl; straight or branched chain alkoxy preferably of 1-4 carbon atoms such as methoxy, ethoxy, propoxy and butoxy; mono- or di-alkylamino such as methylamino, dimethylamino, ethylamino, methylethylamino; alkoxycarbonyl preferably of 2 to 4 carbon atoms such as methoxycarbonyl, ethoxycarbonyl; alkanoyl preferably of 2 to 4 carbon atoms such as acetyl, propionyl; thioalkyl such as MeS-; halo such as fluorine, chlorine and bromine.

Preferred values for R^1 are C_1-C_6 alkyl, C_1-C_6 haloalkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, or a group selected from phenyl, 4-phenylazaphenyl, thienyl, quinolinyl and isoquinolinyl, each optionally substituted as described above, or R^1 is $NHCO_2R^2$ where R^2 is as defined above.

This invention also provides processes for preparing the compounds of formula I. The compounds are prepared by one of the following processes:

a) reacting rapamycin with a compound of formula

$$R^1SO_2hal \qquad (II)$$

or

$$(R^1SO_2)_2O \qquad (III)$$

wherein R^1 is as defined above and hal is a halogen such as chlorine or bromine;

b) reacting rapamycin with a compound of formula

$$R^2OCONSO_2N^{\ominus}{}^{\oplus}(alkyl)_3 \qquad (IV)$$

wherein R^2 is as defined above to give a corresponding compound of formula I where R^1 is $NHCO_2R^2$ where R^2 is as defined above; and if desired after each of the abovementioned processes isolating the compound of formula I in the form of a pharmaceutically acceptable salt thereof.

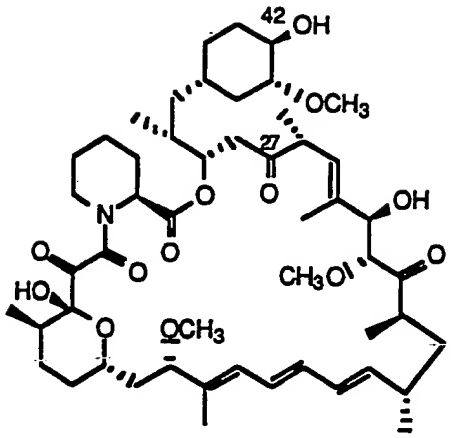
Examples of alkyl in the compound of formula IV are groups having 1 to 6 carbon atoms e.g. methyl or ethyl.

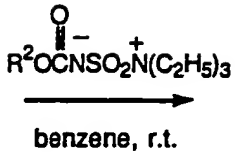
The above processes are described below with reference to various schemes and examples. The schemes illustrate methods which are generally applicable to the preparation of other compounds of the invention. Starting materials not specifically exemplified are known in the art or may be prepared by analogous methods from known starting materials.

The rapamycin 42-sulfonates of this invention can be prepared by the standard literature procedure as outlined below.

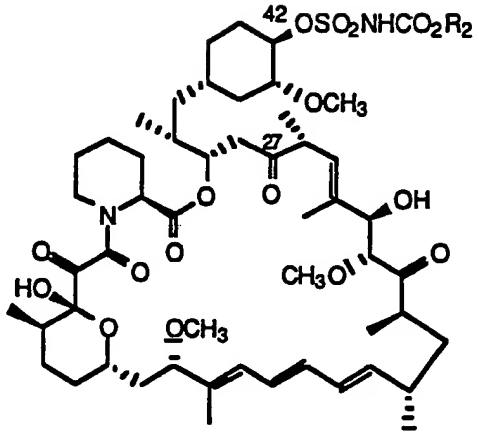
The sulfonate formation between alcohol and sulfonyl halide has been described - see for example Jerry March, Advanced Organic Chemistry, 3rd edition, published in 1985, page 444. The preferred reaction conditions employed in this invention were developed by S Rakhit of Ayerst Laboratories and reported in US Patent 4,316,855 (23 February 1982).

The 42-(N-carboalkoxy)sulfamates of the present invention can also be prepared by reaction of rapamycin with an alkyl(carboxysulfamoyl)triethylammonium hydroxide inner salt (Burgess Salts; see G. M. Atkins Jr. and E. M. Burgess, J. Am. Chem. Soc., 90, 4744, 1968; E. M. Burgess, H.R. Penton Jr. and E. A. Taylor, J. Org. Chem. 38, 26, 1978).





rapamycin



wherein R^2 is as defined above.

The pharmaceutically acceptable salts may be formed from inorganic cations such as sodium, potassium, and the like.

This invention also provides a pharmaceutical composition comprising a compound of formula I or a pharmaceutically acceptable salt thereof and pharmaceutically acceptable carrier.

The following examples illustrate the preparation of representative compounds of this invention.

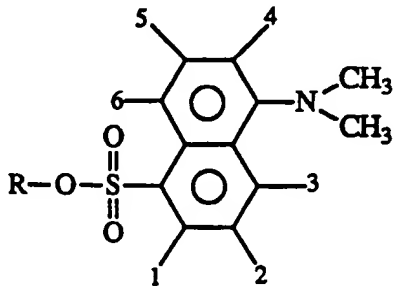
Example 1

Rapamycin 42-ester with 5-(dimethylamino)-1-naphthalensulfonic acid

A solution of 200 mg of rapamycin in 2 mL of pyridine was treated at 0°C under anhydrous conditions with 840 mg of Dansyl chloride and stirred at room temperature for 24 hours. The reaction mixture was diluted at 0°C with 30 mL of 2N HCl and extracted with ethyl acetate. The ethyl acetate extract was washed with brine, dried with $MsSO_4$ and evaporated. The residue was chromatographed on silica gel. Elution with 25% ethyl acetate in benzene afforded 150 mg of the title product as a light yellow powder, m.p. 101-104°C.

IR: 3430 (OH), 1740 (sh), 1720 (both C=0), 1650 (amide C=0), 1450, 1355, 1170 (sulfonate), 1100, 985, 960 cm^{-1}.

^1H NMR ($CDCl_3$, 400 MHz) δ 8.58 (d, 1H, H_1), 8.32 (d, 1H, H_3), 8.25 (m, 1H, H_2), 7.53 (m, 2H, H_5 and H_6), 7.19 (d, 1H, H_4), 3.31, 3.13, 2.72 (all s, 3H, -O-CH_3), 2.79 (s, 6H, $-N(CH_3)_2$) ppm.



MS (neg. ion FAB) 1146 (M^-), 912, 590, 250.

Example 2

Rapamycin 42-ester with 4-methylbenzenesulfonic acid

A solution of 6.0 g p-toluenesulfonyl chloride in 25 mL pyridine was added to a solution of 10.0 g rapamycin at 0°C and the resulting solution was stirred at 20°C for 22 hours. Cold 2N HCl (240 mL) was added and the product was extracted into ethyl acetate, washed with brine, dried over $MgSO_4$ and evaporated to a yellow solid. Chromatography on silica gel eluted with 20% ethyl acetate in methylene chloride afforded 5.3 g product as a white solid, m.p. 108-116°C.

IR(KBr): 3410, 2910, 1710, 1640, 1440, 1160 and 655 cm^{-1}.

NMR ($CDCl_3$, 400 MHz): δ 7.80 (d, 2H, aromatic), 732 (d, 2H, aromatic), 3.33 (s, 3H), 3.14 (s, 3H), 3.13 (s, 3H), 2.44 (s, 3H).

MS (neg. ion FAB): 1067 (M^-), 590, 171, 155.

Example 3

Rapamycin 42-ester with 2-thiophenesulfonic acid

A solution of 0.18 g rapamycin and 0.13 g 2-thiophenesulfonyl chloride in 2 mL pyridine was heated at 55°C for 4 hours, then cooled to 20°C and treated with 40 mL 1N HCl. The product was extracted into ethyl acetate, washed with brine, dried over $MgSO_4$ and stripped of solvent. Chromatography on silica gel eluted with 20% ethyl acetate in methylene chloride afforded 40 mg title compound as a white solid, m.p. 114-119°C.

IR (KBr): 3420, 2915, 1712, 1644, 1440, 1365, 1170 and 660 cm^{-1}.

NMR ($CDCl_3$, 400 MHz): δ 7.67 (1H, aromatic), 7.62 (1H, aromatic), 7.07 (1H, aromatic), 3.29 (s, 3H, OCH_3), 3.14 (s, 3H, OCH_3), 3.09 (s, 3H, OCH_3).

MS (neg. ion FAB): 1059 (M^-), 912, 590, 163.

Example 4

Rapamycin 42-ester with 4-[[4-(dimethylamino)phenyl]azo]-benzenesulfonic acid

Dabsylchloride (0.83 g) was added to a solution of 0.54 g rapamycin in 30 mL dry pyridine and the solution heated at 65-70°C for 24 hours. Upon cooling, the reaction mixture was partitioned between 200 mL 2N HCl and 50 mL ethyl acetate. The product was dried over $MgSO_4$, stripped of solvent and chromatographed on silica

gel eluted with 30% ethyl acetate in methylene chloride, to afford the title compound as a bright red solid, m.p. 118-133°

IR (KBr): 3430, 2930, 1720, 1600, 1360, 1142, 683 and 602 cm^{-1}.

NMR ($CDCl_3$, 400 MHz): δ 8.00 (2H, aromatic), 7.93 (4H, aromatic), 6.76 (2H, aromatic), 3.33 (s, 3H, OCH_3), 3.135 (s, 3H, OCH_3), 3.126 (s, 3H, OCH_3).

MS (pos. FAB): 1223 (MNa^+), 1169, 1137, 918, 306.

Example 5

Rapamycin 42-ester with 1-naphthalene sulfonic acid

1-Naphthalenesulfonyl chloride (0.48 g, mmol) was added to a solution of (0.54 g, mmol) rapamycin in 11 mlL pyridine and the resulting solution was stirred at 20°C for 44 hours. Cold 2N HCl (75 mL) was added and the product was extracted into ethyl acetate, washed with brine, dried over $MgSO_4$ and evaporated to a tan solid. Chromatography on slica gel eluted with 20% ethyl acetate in methylene choride yielded 30 mg product as a white solid, m.p. 110-131°C.

IR (KBr): 3440, 2925, 1720, 1645, 1450, 1175 and 765 cm^{-1}.

NMR ($CDCl_3$, 400 MHz): δ 8.65 (1H), 8.26 (1H), 8.10 (2H), 7.93 (1H), 7.70 (1H), 7.62-7.53 (complex, 2H), 3.32 (s, 3H, OCH_3), 3.13 (s, 3H, OCH_3), 2.64 (s, 3H, OCH_3).

MS (neg. FAB): 1103 (M^-), 912, 590.

Example 6

Rapamycin 42-ester with 8-quinolinesulfonic acid

A solution of (0.30 g, mmol) rapamycin and (0.29 g, mmol) 8-quinolinesulfonyl chloride in 5 mL pyridine was stirred at 20°C for 24 hours. The reaction mixture was partitioned between 2N HCl (10 mL) and ethyl acetate.

The organic layer was washed with brine, dried over $MgSO_4$, stripped of solvent and chromatographed on silica gel eluted with 30% ethyl acetate in methylene chloride, affording 130 mg of title compound as a white solid, mp 120-165°C.

IR (KBr): 3430, 2925, 1715, 1640, 1170, 985 and 785 cm^{-1}.

NMR ($CDCl_3$, 400 MHz): δ 9.18 (1H), 8.49 (1H), 8.25 (1H), 8.09 (1H), 7.65 (1H), 7.55 (1H), 3.32 (s, 3H, OCH_3), 3.13 (s, 3H, OCH_3), 2.60 (s, 3H, OCH_3).

MS (neg. FAB): 1104 (M^-), 912, 590, 208.

Example 7

Rapamycin 42-methanesulfonate, hemiethylacetate, hemihydrate

Under an atmosphere of nitrogen, an ice cold solution of rapamycin (0.46 g, 0.5 mmol) and triethyl amine (0.14 mL, 1.0 mmol) in 5 mL of dry CH_2Cl_2 was treated dropwise with methanesulfonyl chloride (0.943 mL, 0.55 mmol). The ice bath was removed and the solution stirred at ambient temperature for one hour. The reaction mixture was diluted with CH_2Cl_2 and washed successively with H_2O and brine. After drying (Na_2SO_4), the solvent was removed in vacuo to give a yellow foam. Purification by flash chromatography (silica Merck 60, ethyl acetate-hexane 1:1) afforded the title compound (0.37 g, 75% white solid).

NMR (400 MHz, $CDCl_3$): δ 1.654 (s, 3H, $CH_3C=C$), 1.747+1.7502 (2s, 3H, $CH_3C=C$), 3.059 (s, 3H, CH_3SO_2), 3.137 (s, 3H, OCH_3), 3.338 (s, 3H, OCH_3), 3.4003 (s, 3H, OCH_3).

MS (neg. ion FAB, m/z): 991 $(M)^-$, 590, 399.

Anal. calc'd for $C_{52}H_{81}NO_{15}S+0.5\ H_2O+0.5\ C_4H_8O_2$: C, 62.05; H, 8.29; N, 1.34

Found: C, 61.63; H, 8.34; N, 1.49.

Example 8

Rapamycin 42-(2,2,2-trifluoroethane sulfonate), dihydrate

Under an atmosphere of nitrogen, a solution of rapamycin (0.46 g, 0.5 mmol) and triethylamine (0.15 mL, 1.1 mmol) in 2 mL of dry CH_2Cl_2 was treated in one portion with 2,2,2-trifluorothane sulfonyl chloride (0.06 mL, 0.55 mmol). The solution was stirred overnight at ambient temperature. The solvent was evaporated in vacuo to give a yellow foam. The crude product mixture was purified by MPLC (silica Lichrosorb 60, Merck 440*37 mm, ethyl acetate-hexane 1:2, flowrate 20 mL/min) to give the title compound.

NMR (400 MHz, $CDCl_3$): δ 1.656 (s, 3H, $CH_3C=C$), 1.7499+1.7528 (2s, 3H, $CH_3C=C$), 3.138 (s, 3H, OCH_3), 3.341 (s, 3H, OCH_3), 3.377 (s, 3H, OCH_3).

MS (neg. ion FAB, m/z): 1059 $(M)^-$, 590, 560, 427, 163.

Anal. calc'd for $C_{52}H_{81}NO_{15}S+2\ H_2O$: C, 58.02; H, 7.72; N, 1.28

Found: C, 57.94; H, 7.96; N, 1.22.

Example 9

42-O-[[(Methoxycarbonyl)]amino]sulfonyl]rapamycin

Under anhydrous conditions, a solution of rapamycin (0.5 g, 0.55 mmol) and methyl(carboxysulfamoyl)triethylammonium inner salt (0.25 g, 1.2 mmol, prepared as described by Burgess et al., J. Org. Chem. 38, 26, 1978) in 5 mL of benzene was stirred at ambient temperature overnight. The reaction mixture was then diluted with EtOAc (50 mL) and the solution was washed with water and brine and dried (Na2SO4). Removal of the solvent in vacuo yielded an off-white solid which was further purified by MPLC (silica Merck 60 Lichroprep, 440*37 mm, ethyl acetate-hexane 2:1 ----► methanol) to provide the title product as a yellow solid (0.247 g, 43%).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.655 (s, 3H, CH_3C=C), 1.78 (s, 3H, CH_3C=C), 3.13 (m, 3H, CH_3O), 3.39 (m, 6H, CH_3O), 3.71 (s, 3H, CO_2CH_3).

MS (neg. ion FAB, m/z): 1050 (M-H)-.

The comitogen-induced thymocyte proliferation procedure (LAF) was used as an in vitro measure of the immunosuppressive effects of representative compounds. Briefly, cells from the thymus of normal BALB/c mice were cultured for 72 hours with PHA and IL-1 and pulsed with tritiated thymidine during the last six hours. Cells are cultured with and without various concentrations of rapamycin, cyclosporin A, or test compound. Cells are harvested and incorporated; radioactivity is determined. Inhibition of lymphoproliferation is assessed in percent change in counts per minute from non-drug treated controls. The results are expressed by the following ratio:

$$\frac{^3\text{H-control thymus cells - H}^3\text{-rapamycin-treated thymus cells}}{^3\text{H-control thymus cells - H}^3\text{-test compound-treated cells}}$$

A mixed lymphocyte reaction (MLR) occurs when lymphoid cells from genetically distinct animals are combined in tissue culture. Each stimulates the other to undergo blast transformation which results in increased DNA synthesis that can be quantified by the incorporation of tritiated thymidine. Since stimulating a MLR is a function of disparity at Major Histocompatibility antigens, an in vivo popliteal lymph node (PLN) test procedure closely correlates to host vs. graft disease. Briefly, irradiated spleen cells from BALB/c donors are injected into the right hind foot pad of recipient C3H mice. The drug is given daily, p.o. from Day 0 to Day 4. On Day 3 and

Day 4, tritiated thymidine is given i.p., b.i.d. On Day 5, the hind popliteal lymph nodes are removed and dissolved, and radioactivity counted. The corresponding left PLN serves as the control for the PLN from the injected hind foot. Percent suppression is calculated using the non-drug treated animals as allogenic control. Rapamycin at a dose of 6 mg/kg, p.o. gave 86% suppression, whereas cyclosporin A at the same dose gave 43% suppression. Results are expressed by the following ratio:

$$\frac{^3\text{H-PLN cells control C3H mouse} - ^3\text{H-PLN cells rapamycin-treated C3H mouse}}{^3\text{H-PLN cells control C3H mouse} - \text{3H-PLN cells test compound-treated C3H mouse}}$$

The second in vivo test procedure is designed to determine the survival time of pinch skin graft from male DBA/2 donors transplanted to male BALB/c recipients. The method is adapted from Billingham R.E. and Medawar P.B., J. Exp. Biol. 28:385-402 (1951). Briefly, a pinch skin graft from the donor is grafted on the dorsum of the recipient as a homograft, and an autograft is used as control in the same region. The recipients are treated with either varying concentrations of cyclosporin A as test control or the test compound, intraperitoneally. Untreated recipients serve as rejection control. The graft is monitored daily and observations are recorded until the graft becomes dry and forms a blackened scab. This is considered as the rejection day. The mean graft survival time (number of days ± S.D.) of the drug treatment group is compared with the control group.

BIOLOGICAL DATA

Table 1 Biological Activity

Example	LAF Assay (R/A ratio)	PLN (R/A ratio)	Skin Graft Model (days + SD)
1	0.26	-	8.0 ± 0.9
2	0.21	-	8.7 ± 1.2
3	0.23	1.23 (i.p.)	9.3 ± 0.8
4	0.03	-	-
5	0.19	0.92 (i.p.)	9.5 ± 0.3
6	1.32	0.08 (i.p.)	10.7 ± 2.1

	7	1.70	0.36 (i.p.)	9.83 ± 0.98
5	8	0.85	0.83 (i.p.)	10.0 ± 1.4
	9	0.01	0.93 (i.p.)	10.33 ± 0.24

10

The results of these standard pharmacological test procedures demonstrate high immunosuppressive activity both in vitro and in vivo for the compounds of the present invention. A positive ratio in the LAF and PLN test procedures indicates suppression of T-cell proliferation. As transplanted pinch skin grafts are typically rejected within 6-7 days without the use of an immunosuppressive agent, the substantial increase in survival time of the skin grant when treated with the compounds of the present invention further demonstrate their utility as immunosuppressive agents.

Based on the results of these standard pharmacological test procedures, the compounds of this invention are useful in the prevention and treatment of transplant rejection such as heart, kidney, liver, bone marrow, and skin transplants; graft versus host disease; autoimmune and proliferative diseases such as, systemic lupus erythematosus, rheumatoid arthritis, type 1 diabetes, multiple sclerosis, glomerular nephritis, Hashimoto's thyroiditis, myastenia gravis, uveitis and psoriasis; diseases of inflammation such as dermatitis, eczema, seborrhea and inflammatory bowel disease; and fungal infections.

The compounds may be administered neat or with a pharmaceutical carrier to a mammal in need thereof. The pharmaceutical carrier may be solid or liquid.

A solid carrier can include one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the carrier is a finely divided solid which is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, methyl cellulose, sodium carboxymethyl cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins.

Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration include water (partially

containing additives as above, e.g. cellulose derivatives, preferably sodium carboxymethyl cellulose s lution), alcohols (including mon hydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carriers are useful in sterile liquid form compositions for parenteral administration. The liquid carrier for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellent.

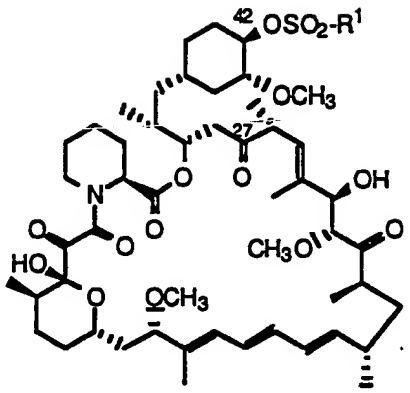
Liquid pharmaceutical compositions which are sterile solutions or suspensions can be utilized by, for example, intramuscular intraperitoneal or subcutaneous injection. Sterile solutions can also be administered intravenously. The compound can also be administered orally either in liquid or solid composition form.

Preferably, the pharmaceutical composition is in unit dosage form, e.g., as tablets or capsules. In such form, the composition is sub-divided in unit dose containing appropriate quantities of the active ingredient; the unit dosage forms can be packaged compositions, for example, packeted powders, vials, ampoules, prefilled syringes or sachets containing liquids. The unit dosage form can be, for example, a capsule or tablet itself, or it can be the appropriate number of any such compositions in package form. The dosage to be used in the treatment must be subjectively determined by the attending physician.

CLAIMS

What is claimed is:

1. A compound of formula (I)



(I)

where R^1 is alkyl, alkenyl, or alkynyl containing 1 to 6 carbon atoms; or an aromatic moiety selected from phenyl, naphthyl and 4-(phenylaza) phenyl wherein said aromatic group is optionally substituted by one or more substituents selected from C_1-C_6 alkyl, C_1-C_6 alkoxy, hydroxy, amino, mono- or di-(C_1-C_6)alkylamino, carboxy, (C_1-C_6 alkoxy)carbonyl, C_2-C_7 alkanoyl, (C_1-C_6) thioalkyl, halo, cyano, nitro, trifluoromethyl, trifluoromethoxy or R^1 is a heteroaromatic group of 5 to 10 ring atoms containing oxygen, nitrogen and/or sulfur as heteroatom(s) wherein said heteroaromatic group is optionally substituted by one or more substituents selected from C_1-C_6 alkyl, C_1-C_6 alkoxy, hydroxy, amino, mono- or di-(C_1-C_6)alkylamino, carboxy, (C_1-C_6 alkoxy)carbonyl, C_2-C_7 alkanoyl, (C_1-C_6) thioalkyl, halo, cyano, nitro, trifluoromethyl, trifluoromethoxy or R^1 is $NHCO_2R^2$ wherein R^2 is lower alkyl containing 1 to 6 carbon atoms; or a pharmaceutically acceptable salt thereof.

2. A compound of claim 1 wherein R^1 is C_1-C_6 alkyl, C_1-C_6 haloalkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl; or an aromatic moiety selected from phenyl, naphthyl and 4-(phenylaza)phenyl wherein said aromatic group is optionally substituted by one or more substituents selected from C_1-C_6 alkyl, C_1-C_6 alkoxy, hydroxy, amino, mono- or di(C_1-C_6) alkylamino, carboxy, (C_1-C_6 alkoxy)carbonyl, C_2-C_7 alkanoyl, thioalkyl (C_1-C_6), halo, cyano, nitro, trifluoromethyl, trifluoromethoxy or R^1 is a heteroaromatic moiety selected from thienyl, quinolinyl and isoquinolinyl wherein said heteroaromatic group is optionally substituted by one or more substituents selected from C_1-C_6 alkyl, C_1-C_6 alkoxy, hydroxy, amino, mono- or di(C_1-C_6) alkylamino, carboxy, (C_1-C_6 alkoxy)carbonyl, C_2-C_7alkanoyl, thioalkyl (C_1-C_6), halo, cyano, nitro, trifluoromethyl, trifluoromethoxy or R^1 is $NHCO_2R^2$ wherein R^2 is lower alkyl containing 1 to 6 carbon atoms or a pharmaceutically acceptable salt thereof.

3. A compound of claim 1 wherein R^1 is 5-(dimethylamino)-1-naphthyl or a pharmaceutically acceptable salt thereof.

4. A compound of claim 1 wherein R^1 is 4-methylphenyl or a pharmaceutically acceptable salt thereof.

5. A compound of claim 1 wherein R^1 is 2-thiophenyl or a pharmaceutically acceptable salt thereof.

6. A compound of claim 1 wherein R^1 is 4-[[4-(dimethylamino)phenyl]aza]phenyl or a pharmaceutically acceptable salt thereof.

7. A compound of claim 1 wherein R^1 is 1-naphthyl or a pharmaceutically acceptable salt thereof.

8. A compound of claim 1 wherein R^1 is 8-quinolinyl or a pharmaceutically acceptable salt thereof.

9. A compound of claim 1 wherein R^1 is methyl or a pharmaceutically acceptable salt thereof.

10. A compound of claim 1 wherein R^1 2,2,2-trifluoroethyl or a pharmaceutically acceptable salt thereof.

11. A compound of Claim 1 wherein R^1 is [methoxycarbonyl]amino or a pharmaceutically acceptable salt thereof.

12. A process for preparing a compound of formula I as shown and defined in Claim 1, which comprises:

a) reacting rapamycin with a compound of formula

$$R^1SO_2hal \qquad (II)$$

or

$$(R^1SO_2)_2O \qquad (III)$$

wherein R^1 is as defined in Claim 1 and hal is a halogen;

b) reacting rapamycin with a compound of formula

$$R^2OCONSO_2N^-(alkyl)_3\,^+ \qquad (IV)$$

wherein R^2 is as defined in Claim 1 to give a corresponding compound of formula I where R^1 is $NHCO_2R^2$ where R^2 is as defined above and if desired after each of the abovementioned processes isolating the compound of formula I in the form of a pharmaceutically acceptable salt thereof.

13. A pharmaceutical composition comprising a compound of formula I or a pharmaceutically acceptable salt thereof as claimed in Claim 1 and a pharmaceutically acceptable carrier.

14. A method of treating transplantation rejection, host versus graft disease, autoimmune diseases, and diseases of inflammation in a mammal by administering an effective amount of a compound of formula I as shown and defined in Claim 1.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/01863

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07D498/18; A61K31/445; //(C07D498/18,311:00,273:00, 221:00)

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07D ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	US,A,4 650 803 (V. J. STELLA ET AL) 17 March 1987 cited in the application see claims 1,5	1,13
P,A	EP,A,0 509 795 (AMERICAN HOME PRODUCTS) 21 October 1992 see claims 1,15	1,13

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
16 JUNE 1993	25. 06. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT FFICE	VOYIAZOGLOU D.

Form PCT/ISA/210 (second sheet) (January 1985)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/01863

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claim 14 is directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9301863
SA 71382

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 16/06/93

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-4650803	17-03-87	AU-B-	583439	27-04-89
		AU-A-	6608086	11-06-87
		CA-A-	1273920	11-09-90
		CA-A-	1312076	29-12-92
		DE-A-	3684574	30-04-92
		EP-A,B	0227355	01-07-87
		EP-A-	0429436	29-05-91
		GB-A,B	2183647	10-06-87
		JP-A-	62215592	22-09-87
EP-A-0509795	21-10-92	US-A-	5118678	02-06-92
		US-A-	5194447	16-03-93
		AU-A-	1488092	22-10-92

EPO FORM P0479